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Pre-storage application of 1-methylcyclopropene does not affect the flavour of 'Conference' pears ripened after 8 months of commercial-standard controlled atmosphere storage

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ABSTRACT

Postharvest 1-methylcyclopropene (1-MCP) applications are commercially used on 'Conference' pears to obtain an improved fruit quality after storage for up to 11 months. Treatment with 1-MCP may result in firmer and greener fruit at the end of storage. During subsequent shelf life, 1-MCP treated pears may show slower ripening, including a reduced rate of softening and a reduced production of aroma volatiles. The lower levels of aroma volatiles and consumer complaints of reduced flavour suggest that flavour is negatively affected by 1-MCP treatments, which has raised concern within in the Dutch fruit industry.

In the present study, the effect of pre-storage 1-MCP treatment on post-storage ripening and flavour perception was studied. Untreated and 1-MCP-treated pears (325 nL L^{-1}) were stored for 8 months at -0.8 °C under controlled atmosphere conditions of 3 kPa O_2 and 0.6 kPa CO_2 according to commercially used protocols. At day 7 and 9 of the subsequent shelf life at 10 °C, 1-MCP-treated fruit showed decreased yellowing and ethylene production, whereas firmness was similar to that of untreated fruit. The production of aroma volatiles was significantly reduced in 1-MCP-treated fruit; this was especially observed for different acetate esters, ethanol and butanol. Despite the reduction in aroma volatiles, a consumer panel could not distinguish (in a Tetrad test) between samples from untreated and 1-MCP-treated fruit with similar firmness. This indicates that the important aroma volatiles, although reduced in abundance, were still above threshold levels and did not affect overall flavour perception. We conclude that 1-MCP does not affect flavour when pears within equal firmness classes are compared.

1. Introduction

The 'Conference' pear (*Pyrus communis* L. cv. Conference) is the most cultivated pear cultivar in Europe (Hendges et al., 2018; Saquet, 2018). This cultivar is renowned for its pleasant aroma, juicy flesh, sweet flavour and buttery texture when ripe (Chiriboga et al., 2013a,2013b; Saquet, 2018). Commercially, to delay their ripening and extend their availability, 'Conference' pears are either stored around -0.5 °C for up to 3 months under regular atmosphere (RA) conditions or for up to 11 months under controlled atmosphere (CA) storage at 3 kPa O_2 and 0.6 kPa CO_2. In northwest Europe, to reduce internal browning disorders, Conference pears are subjected to a RA period of 3–4 weeks prior to CA-storage (Höhn et al., 1996; Verlinden et al., 2002; Rizzolo et al., 2005).

Since pear is a climacteric fruit, ripening depends on the autocatalytic production of ethylene and its binding to an ethylene receptor (Barry and Giovannoni, 2007). The compound 1-methylcyclopropene (1-MCP) is able to block the ethylene receptor and thereby reduces the climacteric peak (Blankenship and Dole, 2003; Watkins, 2006; Chiriboga et al., 2013a,2013b). As a result, treatment with 1-MCP reduces the production of ethylene and other volatiles, respiration, peel colour development from green to yellow and the rate of softening (Watkins, 2006; Chiriboga et al., 2013a,2013b; Hendges et al., 2018). With these effects, 1-MCP treatment can reinforce the effects of CA storage (Rizzolo et al., 2005).

Duration of the effectivity of 1-MCP depends on many factors, such as variety, harvest maturity, dosage, storage bin material, time of treatment and storage conditions (Sozzi and Beaudry, 2007; Chiriboga

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et al., 2013a,2013b). As such, the effect of 1-MCP on reduced softening of pears can last over 6 months of storage (Neuwald et al., 2015; Hendges et al., 2018), but may eventually diminish after prolonged storage (Argenta et al., 2003; Rizzolo et al., 2005). Similarly, the production of volatile compounds and development of flavour can be suppressed over a long period (Hendges et al., 2018), but can recover to a certain extent (Rizzolo et al., 2005; Moya-León et al., 2006). However, recovering the capacity to ripen after 1-MCP treatment and prolonged storage does not always occur (Chiriboga et al., 2011, 2013a,2013b; Chiriboga et al., 2013a,2013b).

Ripening-associated characteristics such as softening, colour changes, sweetening and aroma development are important contributors to flavour and, with that, to consumer acceptance of pears (Kappel et al., 1995; Moya-León et al., 2006). Since 1-MCP can limit the development of these characteristics, there have been concerns within the Dutch pear-sector about the eating quality of 1-MCP treated CA-stored 'Conference' pears.

Earlier reports on the subject have revealed differences in flavour between untreated and 1-MCP-treated pears that vary from improvements in flavour (Gamrasni et al., 2010; Escribano et al., 2016) to reductions in the quality of sensory characteristics such as aroma, taste and firmness (Rizzolo et al., 2005; Moya-León et al., 2006). The sensory term flavour is composed of various sensory characteristics, including aroma, taste and texture, as well as the interactions between them (Noble, 1996; Bult et al., 2007; Kader, 2008). Because of the interactions between flavour attributes, it is important to be able to separate them, in order to assess if, and why flavour and eating quality are affected.

In ripe fruit, differences in aroma occur due to differences in the production of volatile organic compounds (VOCs) such as esters and alcohols (Young et al., 1996; Ulrich et al., 1997; Goff and Klee, 2006; Soukoulis et al., 2013). Such differences can effectively be assessed using Proton Transfer Reaction - Time of Flight – Mass Spectrometry (PTR-ToF-MS), which can rapidly and non-destructively assess the volatile release of a variety of produce, including fresh fruit (Cappellin et al., 2012; Yener et al., 2014; Taiti et al., 2017; Silvis et al., 2019). Differences in taste are most often assessed using descriptive methods in which trained panellists quantitatively determine sensory attributes (O'Sullivan, 2017). However, when no sensory specification is required, more basic difference methods such as the 'unspecified Tetrad test' provide more statistical power (Ennis and Jesionka, 2011; O'Mahony, 2013). Unspecified tests are excellent to determine differences in 'eating quality' and flavour as a whole. However, to pinpoint whether differences in flavour are due to changes in aroma, taste or texture, additional measurements of at least two of these flavour characteristics are required. Texture is a very strong contributor to flavour perception and, therefore, differences in texture are best avoided when studying flavour. Earlier studies involving 1-MCP have avoided textural differences by using different ripening durations (Escribano et al., 2016), or by allowing the effects of 1-MCP to diminish (Argenta et al., 2003; Rizzolo et al., 2005).

In this study, we investigated whether a commercially applied 325 nL L^{-1} post-harvest 1-MCP treatment affects the eating quality of 'Conference' pears after 8 months of storage under commercially applied CA-conditions of 3 kPa O_2 and 0.6 kPa CO_2. Fruit quality was assessed after ripening the fruit to eating ripe firmness classes between 20 and 30 N. Since the use of 1-MCP is important to delay softening, yellowing and defect development during storage and subsequent shelf life, knowing whether or not 1-MCP affects pear flavour is of great importance for the Dutch pear sector.

2. Material and methods

2.1. Plant material and storage conditions

Pear (*Pyrus communis* L. cv. Conference) was harvested on the 11th of September 2017 from an orchard in the Netherlands, during the second half of the harvest window. Pear maturity was determined by firmness (55 N), soluble sugars (12.9 %) and starch (8.1 on a 10 point scale). Fruit were transported on the same day to Wageningen Food & Biobased Research (WFBR, Wageningen, the Netherlands) and placed in a refrigerated storage room under regular atmosphere (RA) at -0.5 °C. Seven days later, part of the batch was temporary placed in another storage room at -0.5 °C for treatment with the 1-MCP product SmartFresh™ at the commercially used dose of 325 nL L^{-1} 1-MCP for 24 h (AgroFresh, Philadelphia, PA, USA). Since the pears originated from the second half of the harvest window, four weeks after harvest the untreated pears were transferred to controlled atmosphere (CA, 3 kPa O_2 and 0.6 kPa CO_2) at -0.8 °C. Nine weeks after harvest the 1-MCP treated pears were also transferred to the same CA conditions at -0.8 °C. This longer period under RA for 1-MCP treated fruit was the recommended protocol by AgroFresh for SmartFresh-application to Conference pears. This protocol reduces storage disorders and is common practice in northwest Europe. Both storage and 1-MCP treatment protocols agreed with commercial standards. After a total of 8 months of storage, the pears were transferred from CA to RA conditions at -0.5 °C for 23 d. This period at RA was applied to do preliminary tests on a subset of pears to assess the pear softening rates. Based on this, shelf-life periods of 7 and 9 d of ripening at 10 °C were chosen to establish 2 eat-ripe firmness classes for the sensory test, for each of the two treatments (see also Figure S1). Analyses of fruit quality aspects was done after 7 and 9 d of shelf life.

2.2. Experimental design

In total 1700 pears were stored, of which 1360 pears were aimed to be used for experiments. For this experiment, 500 pears were stored; 250 untreated and 250 treated with 1-MCP. From each of the two treatments, pears were randomly selected for a pre-test (100), physiological and volatile analysis (30) and the taste test (65), avoiding pears with visible symptoms of damage or rot. The pre-test (200 pears total) was done to determine the rate of softening and planning the experiment accordingly. To limit the chance for overshooting the desired eat-ripe firmness classes due to the accelerated softening common in long-term stored pears, ripening was done at 10 °C instead of the conventional 18−20 °C. Based on this pre-test, 7 and 9 days of ripening at 10 °C were determined to yield pears of the desired firmness classes. Subsequently, half of the pears of each treatment were subjected to 7 and half to 9 days of ripening at 10 °C. Physiological and volatile analyses (60 pears total, 15 pears per treatment combination) were all done on the same individual pears in the following order: colour, VOC analysis by PTR-ToF-MS, ethylene production, weight, firmness and sampling for further VOC identification by GC–MS. For the taste test (130 pears total, 30 per treatment combination), pear firmness was determined to select samples from the required firmness classes.

2.3. Weight and firmness

Fruit weight was recorded using a MS6002TS balance (Mettler-Toledo GmbH, Giessen, Germany).

Fruit firmness was assessed from the fruit flesh, after removal of a piece of skin, at the equatorial region of the pear using a Fruit Texture Analyzer (Güss Manufacturing Ltd, Strand, South Africa) with a 8 mm diameter (0.5 cm^2) probe. The trigger threshold was set at 1 N, whereas the measurement speed and distance were 10 mm s^{-1} and 8.9 mm, respectively.

2.4. Volatile organic compound analysis by PTR-ToF-MS

Volatile organic compound (VOC) production was determined by placing individual pears in 10 L high-density polyethylene (HDPE) drums (Engels Logistiek B.V., Eindhoven, the Netherlands) with red rubber septa (Suba-Seal, Sigma-Aldrich) mounted in the lids. Fifteen repetitions per treatment and ripening period were analysed. After

thorough flushing with clean and filtered air for 2 min, drums were closed and moved to the analysis lab. After equal durations of incubation (~3 h), the headspace was sampled using proton transfer reaction coupled with time of flight mass spectrometry (PTR-ToF-MS). The PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) had a drift voltage of 900 V at 60 °C and 3.80 mbar, resulting in an E/N of 121 Td. PTR mass analysis range was 20–512 *m/z*. Drum headspace sampling was done by direct injection into the PTR-ToF-MS drift tube through a heated (110 °C) peek inlet connected to a syringe needle at a flow rate of 60 mL / min. Sampling was done every second for 60 s total; the first 5−10 s consisted of ambient air, followed by 35 s of sample headspace and 20 s of carbon-filtered air. PTR-ToF-MS data was analysed using the program PTRwid (Holzinger, 2015), after which noise reduction was achieved by averaging over 20 consecutive and stable ToF spectra of the same sample, followed by baseline removal. Data were corrected for pear mass and subsequently Log-transformed. An initial PCA analysis revealed a limited explanation of the variance, after which a subset was made based on the significant differences (Student's t-test, $\alpha = 0.05$) between untreated and 1-MCP treated samples on their respective ripening day. A second PCA analysis yielded a subset of 92 peaks for peak identification. Peak identification was done both within PTRwid and tentatively by comparing with GC–MS results from pooled replicate treatment samples and from literature data on Conference pears.

2.5. Ethylene production

Immediately after PTR-ToF-MS sampling, 5 mL of the headspace was sampled for ethylene analysis. Ethylene analysis was done using a Thermo Trace 1300 GC-FID (Interscience, Breda, the Netherlands) mounted with a multiport injection system (Interscience). This system injected 250 µL samples onto a J&W GS-Gaspro column (30 m, ID 0.32 mm, 60 °C) using Helium as carrier gas at 250 kPa pressure. Data processing occurred using Chromeleon 7.2 (Thermo Fisher Scientific, Waltham, MA, USA). Gas partial pressures were converted to moles according to the ideal gas law (de Wild et al., 1999).

2.6. Fruit background colour

Fruit background colour was assessed using image analysis of two opposite sides of the pears. Images were acquired using a RGB camera (MAKO G-192C POE, Allied Vision, Stadtroda, Germany) positioned in a LED light cabinet (Designed by WFBR and build by IPSS Engineering, Wageningen, the Netherlands). The RGB images were calibrated using a 24-patch colour checker card (Color checker classic, X-rite Europe GmbH, Regensdorf, Switzerland). Image analysis was done using multi-threshold colour image segmentation in the HSV colour space using a software tool developed at WFBR (Wageningen, the Netherlands) to single out the pear background and assess its colour values. Colour values were calculated as h_{ab} in the CIELCh colour space. Among these colour values, 180° and 90° represent green and yellow, respectively.

2.7. Volatile identification by SPME/GC-MS

From all 15 fruit per treatment, a 2 cm thick disc was excised from the equatorial region. Pear samples (2 × 2 × 0.5 cm; l x b x h) were cut from the inner cortex tissue present in this disc, excluding the tissue damaged by the firmness measurement. The samples were quickly diced, flash-frozen in liquid nitrogen, pooled per treatment, and stored at –80 °C. Prior to analysis, samples were ground using an IKA® A11 analytical mill (IKA®-Werke GmbH & Co., Staufen, Germany) and 1 g of the frozen powder was weighed into 20 mL crimp cap vials, closed and placed at -80 °C until analysis. Analysis was done using a TriPlus solid-phase microextraction (SPME) autosampler (Thermo, IL, USA) equipped on a gas chromatograph with a mass spectrometric detector (GC–MS; Trace GC Ultra with a DSQ II MS, Thermo). Vials containing frozen sample powder were defrosted at 0 °C and placed at 6 °C prior to incubation. Prior to SPME extraction, samples were incubated at 60 °C for 20 min under 5 s interval agitation. Subsequently, headspace volatiles were extracted by exposure to a Carboxen/PDMS SPME fibre (Supelco, Zwijndrecht, The Netherlands) for 30 min at 60 °C under 5 s interval agitation. Desorption of the fibre was done for 2 min at 300 °C in the GC injection port in PTV split mode. In between samples, starting after desorption, the SPME fibre was regenerated for 38 min at 300 °C. Gas chromatography was done using a ZB 5MD column (30 m x0.25 mm x0.25 µm) and helium as the carrier gas. Both GC and MS temperatures were 300 °C at the interface and source, respectively. The GC temperature profile started at 40 °C for 2 min, increased first to 160 °C at 4 °C/min, then to 300 °C at 20 °C/min and was finally held at 300 °C for 2 min. Data were analysed using Xcalibur (Thermo Fischer).

2.8. Taste panel unspecified tetrad test

Flavour differences between untreated and 1-MCP treated pears were assessed using an 'unspecified Tetrad test' (O'Sullivan, 2017). In this test, a panel was asked to sort 4 samples originating from two different storage histories into 2 groups, based on flavour aspects. In the case no difference could be detected, the assessor was asked to make 2 groups at random.

Sample preparation started by determining the firmness of both untreated and 1-MCP-treated pears to select pears of two eat-ripe firmness classes: 20 to <25 N (class 1) and 25 to <30 N (class 2), from the pears ripened for 9 and 7 d, respectively. Subsequently, pears were washed and dried using paper towels. Washed pears were cut in half over the stem-calyx axis, avoiding the damaged tissue caused by the firmness measurement, followed by removal of the top 5 cm from the stem-end and a second cut over the stem-calyx axis to yield 4 parts per pear. From these parts, the skin and core were removed and the resulting samples placed in 200 mL transparent polypropylene portion cups and covered with the lids.

Samples from the 4 different variables were provided with 3 digit codes. Six sets of four unique codes were used in total (Table S2). Three of these sets contained pears from firmness class 1 and three from firmness class 2. Each set consisting of a untreated and a 1-MCP-treated sample and was in random order supplied to six panellists, each of which received their samples in the order of tasting along with an evaluation form. The panel consisted of 36 volunteers, recruited amongst employees within WFBR and familiar with the taste of 'Conference' pears. Among these volunteers, 44 and 56 % were male and female, respectively. The average age was 41.2 y. The tasting session took place at their desk and at the panellists' own speed. Tasting took place at room temperature with fruit of room temperature and in between sample tasting participants could drink water to neutralize their pallet.

The Tetrad taste test was designed and the data collected using the program EyeQuestion 4.11.3 (Logic8 BV, Elst, the Netherlands).

2.9. Data and statistical analysis

Physiological data was graphed and statistically analysed (Student's t-test, $\alpha = 0.05$, $n = 15$) using Microsoft Excel. Processed volatile data was transformed (mean-centered and standard deviation-scaled) using Unscrambler X (CAMO Software, Oslo, Norway), which was also used for the PCA-analysis. Tetrad test results were analysed (1-tailed binomial test, $P(\pi_{\text{correct answer}} \leq 0.333, n = 36, \alpha = 0.05)$) using EyeOpenR 4.11.3 (Logic8 BV, Elst, the Netherlands).

3. Results

3.1. Establishing eat-ripe firmness classes of Conference pears

In order to assess whether volatile production and flavour differed between untreated and 1-MCP treated pears, with a minimum influence

of texture, we generated two eat-ripe firmness classes. These eat-ripe firmness classes were generated for both treatments by ripening pears for 7 and 9 d, respectively. To assess the effects of the 1-MCP and ripening treatments on pear physiology, we included ethylene production and background colour measurements. Since volatile production is generally normalized for sample weight, weight was included as well.

From each of the four treatment groups, 15 individual pears were randomly selected and successively analysed for weight, volatile production by PTR-tof-MS, ethylene production, background colour, firmness and volatile identification by GC—MS. Pear weight was used to normalize the volatile data. At 7 and 9 d of ripening, ethylene production was significantly higher in the untreated pears compared to the 1-MCP treated pears (Fig. 1a). The colour of the pears, expressed in degrees of h_{ab}, was significantly more yellow in the untreated pears (Fig. 1b). Between the two days of ripening, the colour did not differ significantly. The average firmness of the untreated and the 1-MCP treated pears was the same for 7 and 9 d of ripening at 28.7 and 20.6 N, respectively (Fig. 1c). These results show that the ripening setup succeeded in establishing two distinct eat-ripe firmness classes that each included both untreated and 1-MCP-treated pears.

3.2. Differences in volatile production due to ripening and treatment with 1-MCP

Volatile production of the individual pears was measured using PTR-ToF-MS and resulted in detecting over 550 peaks in the mass range from m/z 16 – 445. On these peaks and the ethylene production, colour and firmness data, a PCA analysis was done to identify the volatiles contributing to the differences between the untreated and 1-MCP treated pears. Due to noise in the volatile dataset, PCA analysis initially showed a limited explained variance (35 % between ripening days, 11 % between untreated and 1-MCP treated; data not shown). Since we were only interested in the differences between treatments, we analysed a subset consisting of 71 peaks that differed significantly (Student's t-test) between untreated and 1-MCP-treated pears after both 7 and 9 d of ripening and in addition 21 confirmed isotopes were removed. Ethylene production, colour and firmness data were also added to this data set. After PCA analysis, 67 % of the variance was explained by the treatment and 9 % by the days of ripening (Fig. 2a). When looking at the loadings, all volatiles that contributed significantly to the variance between untreated and 1-MCP treated pears were more abundant in the untreated pears (Fig. 2b). Ethylene production, colour and firmness did not contribute significantly to the explained variance.

From the loadings, the volatiles that contributed significantly to the difference between treatments were selected and, after removal of volatile masses with an expression below 1000 cps kg^{-1}, listed for tentative analysis (Table 1).

On the resulting 52 masses, tentative identification was done using data from literature and comparison to GC–MS identification on pooled samples from each of the 4 variables. All identifiable masses related to alcohols, aldehydes and esters. Additionally, 11 of these masses corresponded to fragmentation products of alcohols, aldehydes and esters caused by the H_3O^+-ionisation within the PTR (Soukoulis et al., 2013). These fragments were divided into primary and secondary fragments. Primary fragments, direct reaction products from the ionisation, originated mainly from alcohols and aldehydes containing three or more carbon molecules. Secondary fragments, reaction products from ionisation of primary fragments, originated mainly from esters. Ethanol, though appearing at the expected mass, may also have originated from the fragmentation of ethyl-esters. Additional GC–MS identification allowed us to critically assess the tentative identification of the alcohols and esters and to separate these from the fragmentation-derived products. GC–MS identification confirmed the presence of ethanol and butanol, as well as that of methyl-, ethyl-, propyl-, butyl-, pentyl- and hexyl-acetate esters. Acetaldehyde could not be detected using GC–MS, but their production rates (as measured by PTR-ToF-MS) were amongst the highest of the recorded volatiles. Since acetaldehyde has a unique mass and is not known to be an abundant fragmentation product (Soukoulis et al., 2013), it was considered to be a genuinely produced compound.

3.3. Distinguishing untreated and 1-MCP treated pears based on flavour perception

In order to assess whether the 1-MCP treatment also led to differences in flavour perception, we performed a unspecified Tetrad test using a consumer taste panel. Using such a distinguishing test has the advantage that taste preferences of the panellists do not matter, since the panellists need to match pieces of fruit based on perceived differences and do not necessarily need to describe the differences. Since texture is an important part of the flavour experience, we decided to minimize this factor by selecting two distinct eat-ripe firmness classes. These firmness classes encompassed 25 to <30 and 20 to <25 N from the 7- and 9-day ripened pears, respectively. Panellists were given two samples of untreated pears and two samples of 1-MCP treated pears within the same firmness class, in randomized order. They were asked to sort them into

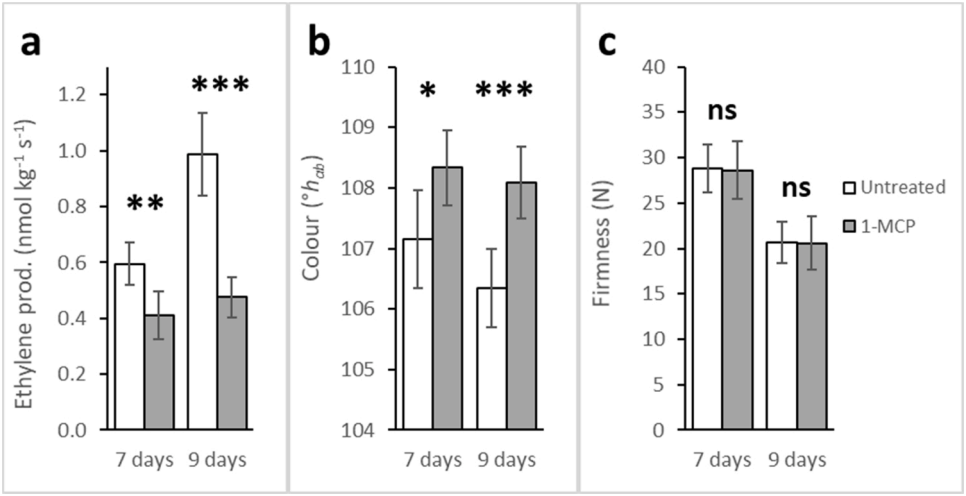

Fig. 1. Characteristics of Conference pears after CA-storage and 7 and 9 d of ripening at 10 °C. Pears were either untreated or treated with 1-MCP before storage. Assessments were done on ethylene production (a), colour (b) and firmness (c). Data are means +/- 95 % CI, n = 15. Statistical notations (Student's t-test) are *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

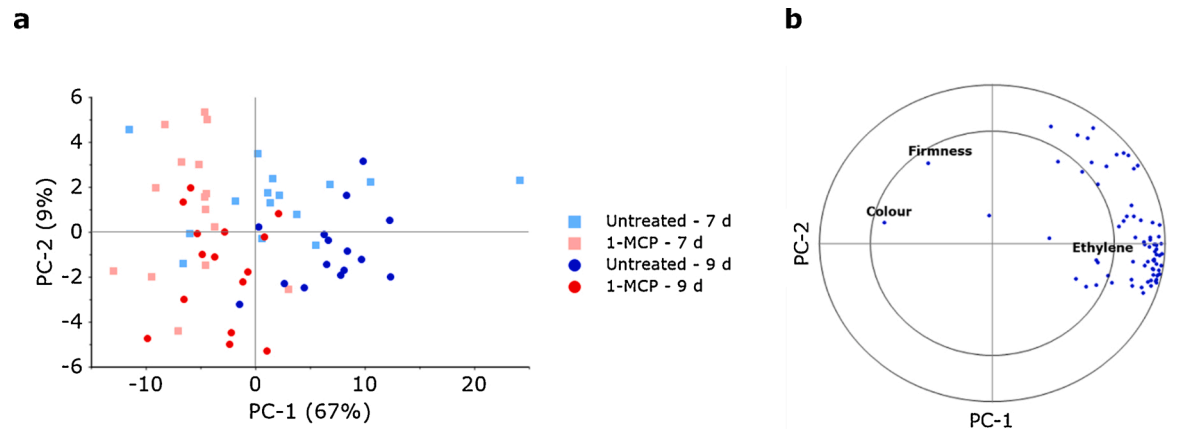

Fig. 2. PCA analysis of PTR-MS and physiological data of Conference pears after CA-storage followed by 7 and 9 d of ripening at 10 °C. PCA analysis (a) was done on ethylene production, colour and firmness data as well as on the volatiles that were significantly different on both ripening days (student's t-test, $p < 0.05$, $n = 15$). Each point represents a single pear that was either untreated (blue) or treated with 1-MCP (red) and ripened for 7 d (square) or 9 days (circle). The loading plot (b) shows the ethylene production, colour, firmness and volatile production data contributing to the principal components in Figure a. Volatile data that significantly contributed to the explained variation is shown between the circles (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

two groups of two samples of similar flavour. All panellists completely filled in the evaluation form. Of the 36 panellists, 14 correctly distinguished between the 1-MCP- and untreated samples, yielding a p-value of 0.293 (Table 2). These results show that there was no significantly noticeable difference in flavour between the untreated and 1-MCP treated Conference pears at equal firmness after long-term storage.

4. Discussion

4.1. Effectiveness of 1-MCP during long-term CA storage

Treatment with 1-MCP has been proven to inhibit the ethylene-dependent enhancement of pear softening, yellowing and the production of ethylene and other volatiles during storage (Watkins, 2006; Chiriboga et al., 2013a,2013b; Hendges et al., 2018), with the added advantage of reducing pathogen infection rates (Spotts et al., 2007). After storage of 8 months, followed by 7 or 9 days of ripening, 'Conference' pears treated with 1-MCP showed several known 1-MCP effects compared to untreated pears. Pear background colour, as determined using image analysis in the CIELCh colour space, was more green in the 1-MCP treated pears (Fig. 1b), which agrees with earlier reports (McGuire, 1992; Rizzolo et al., 2005; Hendges et al., 2018). Ethylene production during ripening was reduced by approximately 50 % in the 1-MCP treated pears (Fig. 1a), which is comparable to other reports (Argenta et al., 2003; Rizzolo et al., 2005). These observations suggest ethylene-dependent ripening to be slowed down in the 1-MCP treated pears, even though the firmness of the untreated and 1-MCP treated pears in our experiment was equal (Fig. 1c). Disappearance over time of 1-MCP related differences in firmness has been observed previously and the effects of 1-MCP have been suggested to dwindle after long-term storage, particularly regarding firmness loss (Argenta et al., 2003; Rizzolo et al., 2005). The activity of polygalacturonase, an important cell-wall degrading enzyme in pears, was recently indicated to be ethylene-independent in 'Blanquilla' pears (Lindo-García et al., 2019). While this may be different in 'Conference' pears, these observations open the discussion on exactly how various cell wall degrading enzymes are regulated by ethylene, 1-MCP and during long term CA-storage in relation to firmness, as this currently seems unclear. With this in mind, the lack of difference in pear firmness in this experiment does not necessarily undermine the effectiveness of the 1-MCP treatment. Over the last decades 1-MCP has proven its value in improving the storability of pears by delaying firmness loss, yellowing and defect development (Watkins, 2006, 2015). However, its use needs optimisation to prevent batches of pears that remain firm with associated taste issues (Chiriboga et al., 2011, 2013a,2013b; Saquet, 2019). While the effects of 1-MCP may decrease when storage surpasses 6–8 months (Argenta et al., 2003; Rizzolo et al., 2005; Moya-León et al., 2006) (Fig. 1), this does not imply that 1-MCP has had no beneficial effects during the first 6–8 months of storage. Since storage losses were not the focus of the present study and there are still residual effects of the 1-MCP treatment, we see no reason to question the effectiveness of the 1-MCP treatment during long-term storage. However, we do see reason to recommend investigating this topic further.

4.2. Treatment with 1-MCP reduces pear volatile production after long-term CA storage

Previous reports on volatile production of stored pears have mainly been based on GC–MS analysis (Argenta et al., 2003; Rizzolo et al., 2005; Moya-León et al., 2006; Hendges et al., 2018). In this work, we have used PTR-ToF-MS to benefit from its sensitivity and sampling speed, which allowed increased sample replication and limited ripening-related volatile dynamics during the measurement window (Soukoulis et al., 2013). Volatile production was shown to be reduced in the 1-MCP-treated pears after both 7 and 9 d of ripening. Our observed reduction in volatile production in the 1-MCP treated pears (Fig. 2b) is similar to that in other reports (Argenta et al., 2003; Rizzolo et al., 2005; Moya-León et al., 2006; Hendges et al., 2018). Despite the variations in 1-MCP concentration, storage conditions, storage times and post-storage ripening conditions used in these works, all show that 1-MCP treatment reduces the levels of individual volatiles. Furthermore, it is noteworthy that in these reports the 1-MCP-treated pears were more firm compared to untreated pears, which could indicate that the reduced volatile production was due to different physiological ripeness stages. Within our dataset this was visible to some extent as well; individual pears with a relatively high volatile production were relatively soft and vice versa (data not shown). Nevertheless, the variation between the individual pears per treatment was smaller than that between the treatments.

While PTR-ToF-MS by itself is not able to distinguish between volatiles with identical molecular masses, this limitation has been resolved using tentative identification from literature reports (Farneti et al., 2015a,2015b; Silvis et al., 2019), gas chromatography (Aprea et al., 2015) and through study of fragmentation patterns of alcohols, esters and aldehydes (Aprea et al., 2007; Soukoulis et al., 2013). In this work, data derived from literature and GC–MS analyses on pooled sample material allowed us to tentatively identify the masses of the more

Table 1
Tentatively identified abundantly and differentially produced volatiles between untreated and 1-MCP-treated pears. Included are volatiles from the loadings shown in Fig. 2b with a volatile production > 1000 cps kg^{-1}, excluding isotopes. Listed are the mass/charge ratio (*m/z*), the corresponding molecular formula (Formula), tentative identification based on literature and the corresponding references (Reference), identification based on GC–MS analyses of pooled samples (GC–MS ID) and additional remarks regarding fragmentation due to ionization within the PTR-TOF-MS (Remarks). Abbreviations: 1, (Rizzolo et al., 2005); 2, (López-Nicolás et al., 2009); 3, (Soukoulis et al., 2013); 4, (Riu-Aumatell et al., 2005); a, primary fragment; b, secondary fragment from esters.

m/z	Formula	Tentative ID	Reference	GC-MS ID	Remarks
27.023	C2H3+				
29.037	C2H5+	alcohol fragment	3		a
41.038	C3H5+	alcohol fragment	3	–	ab
43.018	C2H2OH+	Propanal, ester fragment	1, 3	–	a, b
43.054	C3H7+	Propanol	3	–	ab
45.034	C2H4OH+	Acetaldehyde	1	–	–
47.049	C2H6OH+	Ethanol	1, 4	Y	–
57.069	C4H9+	Butanol, 2-Methylpropanol	1, 2, 3	Y, -	ab, a
60.019					
61.029	C2H4O2H+	acetate ester fragment	3	–	b
63.043	C2H6O2H+	Ethane-diol	–	–	–
69.031	C4H4OH+	Furan	–	–	–
71.049	C4H6OH+	ester fragment, aldehyde fragment	3	–	b
71.086	C5H11+	Pentanol, 2-Methyl-1-butanol	1, 2, 3, 4	–	ab, a
75.044	C3H6O2H+	Methyl acetate	1, 3, 4	Y	–
79.040	C2H6O3H+		–	–	–
83.085	C6H11+	Hexenol	–	–	a
85.101	C6H13+	Hexanol	1, 2, 3, 4	–	ab
87.079	C5H10OH+	Pentanal, 3-Methyl-butanal	4	–	–
88.076					
89.023					
89.060	C4H8O2H+	Ethyl acetate, Butanoate ester fragment	1, 3, 4	Y,-	b
90.019					
92.991					
103.075	C5H10O2H+	Propyl acetate	1, 2	Y	–
117.021					
117.092	C6H12O2H+	Butyl acetate, Ethyl butanoate, 2-Methylpropyl acetate	1, 2, 4	Y, -, -	–
123.116	C9H15+				
131.107	C7H14O2H+	Isoamyl acetate, Pentyl acetate	4	–	–
134.118					
135.107	C6H14O3H+				
136.113					
145.123	C8H16O2H+	Hexyl acetate, Butyl butanoate, Ethyl hexanoate	1, 2, 3, 4	Y, -, -	–
146.082					
148.134	C7H17O2NH+				
162.150	C8H19O2NH+				
163.135	C8H18O3H+				
187.168	C11H22O2H+	Ethyl decadienoate, Pentyl hexanoate, Heptyl isobutyrate, Hexyl 2-methyl butyrate	–	–	–

abundant volatiles that showed significant differences between untreated and 1-MCP treated pears (Table 1).

The identified volatiles were mostly esters and alcohols and some aldehydes; these compounds are well known in relation to pear ripening after long-term CA storage conditions (Argenta et al., 2003; Rizzolo et al., 2005; Moya-León et al., 2006; Hendges et al., 2018). Methanol was the most abundant volatile detected and has been connected to fruit softening, likely as a by-product of the de-methylation of pectin that is required for polygalacturonase to be able to degrade pectin (Chervin et al., 1999; White et al., 2016). While most volatiles showed a 2–3 logarithmic-fold lower production in the 1-MCP treated pears, this fold difference was only 1.4 in the case of methanol (Table 1). This low fold difference in response to 1-MCP is in line with the lack of difference in firmness (Fig. 1c).

Table 2
Tetrad test results of untreated and 1-MCP-treated Conference pears after storage and ripening. Pears were ripened at 10 °C to firmness classes of either 20 to <25 or 25 to <30 N, each of which was equally represented in the test. Statistical abbreviations (1-tailed binomial test, $P(\pi_{correct\ answer} \leq 0.333, n = 36, \alpha = 0.05)$): ns, not significant.

Number of panellists	Number of correct answers	Number of incorrect answers	P	Significance
36	14	22	0.293	ns

In this work, we measured volatile production from intact pears and assumed that the measured differences between untreated and 1-MCP-treated fruit would also be apparent during eating. This, however, is not certain. During eating, different volatiles can have different release patterns prior to and following (artificial) chewing (Farneti et al., 2013, 2015a,2015b). In tomato, this is related to the existence of glyco-conjugates that release phenylpropanoid volatiles after enzymatic cleavage induced by tissue disruption (Tikunov et al., 2010, 2013; Rambla et al., 2014). During mastication of apple, esters, acetaldehyde and some alcohols are rapidly released, but otherwise not influenced, while the release of methanol and ethanol is not directly influenced (Farneti et al., 2015a,2015b). Similar to apples, esters, alcohols and acetaldehyde are the most abundant volatiles in both intact and juiced 'Conference' pears (Rizzolo et al., 2005; López-Nicolás et al., 2009). As such, chewing pears would likely release higher amounts of the esters, alcohols and acetaldehydes than those measured in intact pears (Table 1). Since these volatiles already comprise the significant differences measured between intact untreated and 1-MCP-treated pears, measuring volatiles during chewing would likely have yielded similar conclusions.

When differently treated fruit show differences in aroma volatile production, this does not automatically mean that differences in aroma can be detected by consumers eating the fruit. Volatiles can be perceived in two different ways, through the nasal cavity or via the oral cavity, i.e. by orthonasal and retronasal olfaction (Klee and Tieman, 2013). Since the volatiles detected in this work were produced by intact fruits, they will mainly contribute to the pear aroma that is perceived during orthonasal olfaction, e.g. by smell (Farneti et al., 2013; Klee and Tieman, 2013). Perception of volatiles during orthonasal olfaction furthermore depends on odour detection thresholds (Rizzolo et al., 2005). Factoring in these thresholds, perception of the differences in production of alcohols, acetaldehyde and esters need to exceed values of 500, 198 and 2 μg kg^{-1}, respectively. Since we focused on relative production levels of volatiles between untreated and 1-MCP treated pears, we cannot conclude whether the differences in aroma of intact pears can indeed be perceived by consumers. Comparing the relative differences in production and odour thresholds between the identified esters, acetaldehyde and alcohols, we can conclude that the differences in ethyl acetate will be most perceivable, followed by hexyl acetate and acetaldehyde, and that the differences in alcohols will be less perceivable (Table S1).

4.3. Treatment with 1-MCP does not distinguishably change flavour after long-term CA storage

The differences in colour and volatile-production indicate a residual

effect of 1-MCP on the ripening physiology of the pears, including its aroma production. Aroma is considered a very important contributor to flavour (Noble, 1996; Bult et al., 2007; Kader, 2008). Despite having observed differences in the production of aroma volatiles, 1-MCP-treated 'Conference' pears could not be distinguished from untreated fruit (at equal firmness level) by a consumer panel (Table 2). Earlier reports have mentioned both positive and negative changes in pear flavour after treatment with 1-MCP and these depended likely on cultivar, storage conditions, storage time and ripening duration. Improvements in flavour of 1-MCP treated Bartlett pears have been defined as more sweet, juicy and aromatic in pears that had been ripened shortly after picking (Escribano et al., 2016). Another definition of improved flavour after 1-MCP treatment was defined as a "better overall flavour" in Spadona pears that had been ripened after 6-months storage under CA (Gamrasni et al., 2010). In this last example, untreated pears could be considered very ripe and 1-MCP-treated pears less ripe. Negative effects of 1-MCP treatment involved "poor flavour and texture" in 'Conference' pears at various 1-MCP concentrations and storage durations (Rizzolo et al., 2005) and reduction in flavour at increased texture in 'Packham's Triumph' pears after 2 months of cold storage (Moya-León et al., 2006). Interestingly, already after 4 months of cold storage, untreated and 1-MCP-treated 'Packham's Triumph' pears showed similar texture loss and limited to no differences in flavour attributes (Moya-León et al., 2006). In these reports, the focus was mainly on describing the differences in flavour, which in a number of cases could have been influenced by the differences in firmness levels or effective ripening times (Rizzolo et al., 2005; Moya-León et al., 2006; Gamrasni et al., 2010; Escribano et al., 2016). Such observations, that pear softening rates are similar after long-term storage (Argenta et al., 2003; Rizzolo et al., 2005), allowed us to focus on our main question: whether 1-MCP treated and untreated pears differ in flavour. As such, we decided on a unspecified taste test using a considerable number of experienced consumers of 'Conference' pears. In apple, a similar large consumer response experiment had shown that consumers could distinguish between 1-MCP and untreated Gala apples after 6 months CA-storage at equal firmness (Marin et al., 2009). However, this distinction was based on a triangle-like test in which 2 of the 3 tasted parts were derived from the same apple, which may have skewed the results towards the difference. We avoided such a situation by providing panellists with parts from different pears within a small eat-ripe firmness range (Kappel et al., 1995).

Despite the fact that volatile aroma is considered a very important contributor to flavour (Noble, 1996; Bult et al., 2007; Kader, 2008), the differences in volatile production did not yield a significant difference in flavour (Table 2). Preliminary experiments in our laboratory, using pears with a much higher firmness (55−60 N), showed significant differences in volatile production, but also here a consumer panel could not distinguish between the untreated and 1-MCP treated fruit. (data not shown).

This is interesting as esters are well known to contribute to fruity aromas (Lara et al., 2003; Moya-León et al., 2006) and these aromas are known to enhance taste aspects such as sweetness (Noble, 1996). In order to affect taste perception, aroma volatiles have to be presented retronasally during chewing and swallowing (Bult et al., 2007; Klee and Tieman, 2013). Furthermore, during chewing, both ripening and newly formed volatiles are released from the fruit matrix (Farneti et al., 2013, 2015a,2015b). Pear sample size was sufficiently large to require multiple biting, chewing and swallowing actions and result in retronasal presentation of aroma volatiles. Since odour thresholds for both ortho- and retronasal olfaction are similar in a native fruit matrix (Plotto et al., 2008), in our experiment esters would be expected to dominate the differences between the treatments (Table S1). The fact that a consumer panel could not distinguish between untreated and 1-MCP-treated pears suggests that the production levels of the important aroma volatiles in both groups were well above the threshold levels for flavour perception. A trained expert taste panel may have been better able to define the aromas of ester and newly formed volatiles and thus to distinguish between the two pear treatments. Nevertheless, the results show two things: firstly they illustrate the importance of separating flavour attributes when employing taste tests and secondly they clearly show that consumers familiar with 'Conference' pears could not distinguish between pears that had been exposed to either of the two commercial storage protocols with or without 1-MCP.

5. Conclusions

This investigation suggests that application of 1-MCP and storage for 8 months, according to a commercially recommended storage protocol, has no effect on the flavour of 'Conference' pear when consumed at comparable firmness levels. This information will help the fruit sector in their choice for long-term storage strategy and in addressing complaints regarding flavour. While aroma volatile production was decreased, this did not have a distinguishable influence on the flavour of the pear. Although pear firmness was indistinguishable between 1-MCP treated and untreated pears after 8 months, there is no reason to question the benefits of using 1-MCP for storage periods up to 6–8 months as other quality aspects (e.g. colour) were favourably affected. Further research is recommended to investigate the effectiveness of 1-MCP to maintain pear quality during storage extending past 6–8 months.

CRediT authorship contribution statement

Bastiaan Brouwer: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing. **Manon Mensink:** Conceptualization, Methodology, Validation, Formal analysis, Investigation. **Esther Hogeveen-van Echtelt:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition. **Ernst J. Woltering:** Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.postharvbio.2020.111448.

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